The Role of Leukocyte Specific Protein 1 (LSP1) during HIV-1 Infection in Human T Cells

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by

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Abbreviations

Abbreviations not otherwise specified, in order of appearance, starting with the introduction.

ART – Antiretroviral Therapy
Gag – Group Specific Antigen
Pol – Polymerase
Env – Envelope
Vpr – Viral Protein R
PPT – Polypurine Tract
U3, U5 – Upstream 3,5
V3 – Hypervariable Region 3
CCR5 – CC Chemokine Receptor 5
CXCR4 – CXC Chemokine Receptor 4
Tat – Transactivator of Transcription
Rev – Regulator of Expression of Virion Proteins
Nef – Negative Factor
Vif – Viral Infectivity Factor
Vpu – Viral Protein U
RRE – Rev Response Element
CTL – Cytotoxic T Lymphocyte
MHC – Major Histocompatibility Complex
ER – Endoplasmic Reticulum
Fc – Fragment crystallizable
DC-SIGN – **D**endritic **C**ell-**S**pecific **I**ntercellular adhesion molecule-3-**G**rabbing **N**on-integrin
PKC – Protein Kinase C
shRNA – short hairpin RNA
X4 – short for CXCR4
HTLV – Human T cell Leukemia Virus
R5 – short for CCR5
FBS – Fetal Bovine Serum
RT-PCR – Realtime Polymerase Chain Reaction
GAPDH – Glyceraldehyde 3-phosphate dehydrogenase
CFSE – Carboxyfluorescein succinimidyl ester
ELISA – Enzyme-Linked Immunosorbent Assay
MOI – Multiplicity of Infection
GFP – Green Fluorescent Protein
PBS – Phosphate Buffered Solution
iDC – Immature Dendritic Cell
F-actin – Filament-Actin
GPCR – G Protein-Coupled Receptor

Abstract

The Role of Leukocyte Specific Protein 1 (LSP1) during HIV-1 Infection in Human T-Cells

The Leukocyte Specific Protein 1 (LSP1) gene encodes a filament-actin binding protein that affects leukocyte motility via cytoskeleton remodeling. In dendritic cells, current data shows that LSP1 interacts with DC-SIGN and causes enhanced virus transfer to T-cells, while not typically infecting dendritic cells. However, while its association in several HIV-related signaling pathways has been shown, its primary role during HIV pathogenesis in T-cells is unknown. To explore the potential role of LSP1 during HIV-1 infection in T-cells, LSP1 expression was altered in cell lines susceptible to cytopathic CXCR4-tropic HIV-1 strains, which result in T-cell depletion characteristic of AIDS patients. Two different LSP1 mRNA binding sites were targeted for knockdown (KD) using an shRNA plasmid to transcriptionally silence LSP1 expression in MT4 cells, as well as a non-targeting shRNA control. The relative amount of free virus in the supernatant from infected cells was quantified by ELISA; LSP1-KD MT4 cells showed significant reduction in p24 levels by 3 days post-infection. This result was explored with an LTR-driven GHOST reporter to examine the relative levels of infectious vs. total virus produced. In this assay, LSP1-KD cells showed a significant reduction of infectious virion. An HIV-1 binding and entry assay showed significant reduction in binding in LSP1-KD cells, while the entry assay showed no difference.

These findings suggest that LSP1 knockdown cells are less infectious due to inhibition between the egress and maturation stages of HIV replication. Interestingly, LSP1-KD cells showed lower levels of total HIV infection despite enhanced cell viability, meaning that less infection occurred despite there being more available cells to infect. Additionally, LSP1-KD reduces the infectiousness of HIV-1 virion produced. Therefore, understanding the physiology of LSP1 during HIV-1 pathogenesis could provide novel therapeutic strategies to protect against HIV-1 and enhance T-cell viability.

Introduction

The importance of finding an effective treatment for HIV/AIDS is without question as one of the world's foremost public health crises. The disease known as Acquired Immunodeficiency Syndrome (AIDS) was first recognized as a clinical entity in 1981. By 1986, an international commission officially named the virus that causes AIDS the Human Immunodeficiency Virus (HIV).[1,2] Since the first cases were reported in 1981, more than 25 million people worldwide have died from HIV/AIDS (hereafter referred to collectively as HIV). Currently, there are 33.4 million people living with HIV.[3] HIV is transmitted through the exchange of blood, seminal fluid, vaginal fluid, and breast milk. However, sexual transmission is the primary route, accounting for 75-90% of new infections.[4] Each year, approximately 2 million people die from HIV, while another 2.7 million are newly infected with it. However, while HIV continues to be a global problem, it disproportionally affects more impoverished nations, especially those in Sub-Saharan Africa, where 22.5 million are living with HIV.[3]

Yet, even in wealthy countries like the United States, treatment for the 1.1 million individuals living with HIV treatment carries a substantial economic burden, at an estimated lifetime cost of $380,000 per person ($618,900 for adults who initiate ART with CD4 cell count < 350/mL).[3,5,6] Prevention has helped to reduce HIV prevalence rates in a small but growing number of countries and the number of new HIV infections are believed to be on the decline.[3,5] However, the complexity of HIV leaves great uncertainty in where the next generation of

HIV treatments shall arise. While genetic approaches appear promising, the technical hurdles to be overcome are staggering.[7,8,9]

HIV-1 Structure & Function*,[10]

Life expectancy for individuals living with HIV has improved by an impressive margin (<7 years in 1993, ~10 years in the late 90's, and ~24 years at present).[6] But HIV remains astonishing in how this relatively simple virus has remained incurable for decades despite the emergence of effective drug therapies[9]. The HIV-1 genome codes for fifteen different proteins, using its two identical positive sense (but not mRNA-like) genomic RNA strands **(figure 1)**. Of the nine open reading frames in the genomic RNA, the portions that cause HIV to be classified as a retrovirus are the *gag*, *pol*, and *env* coding regions.

*As this section is not the primary focus of the paper (and for my sanity), unless otherwise noted, references in the HIV-1 Structure & Function section will refer to the review article by Frankel and Young, or be in addition to it.

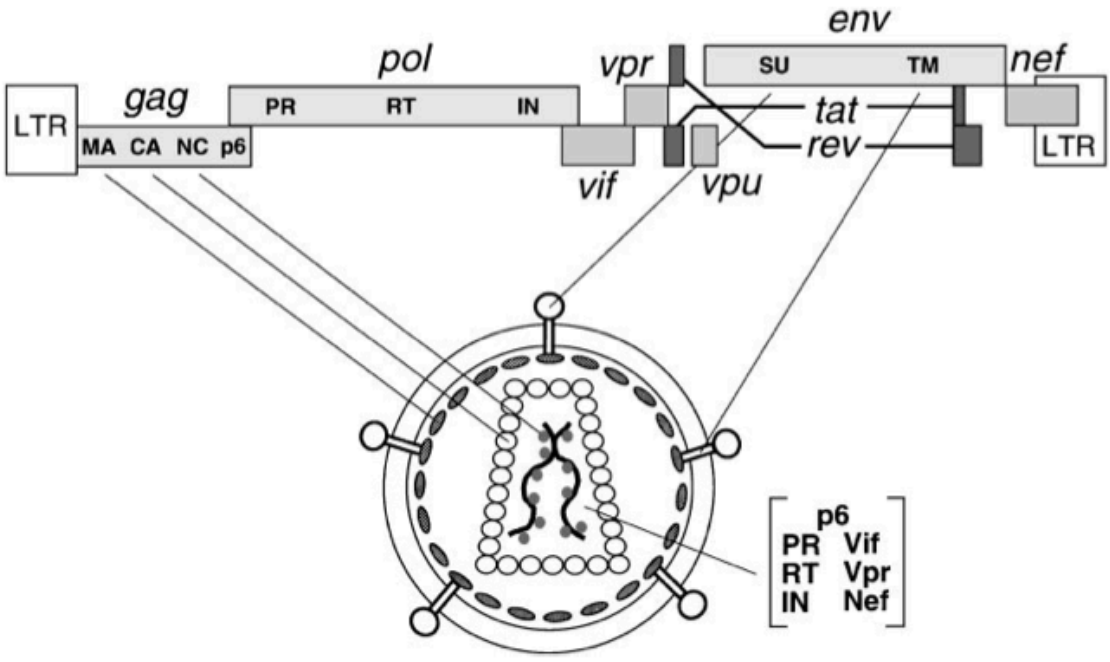


Figure 1 Organization of the HIV-1 genome and virion.
Image from Frankel & Young, 1998.[10]

The *gag* region codes for the (4) core structural proteins of the virus: MA (matrix, p17), CA (capsid, p24), NC (nucleocapsid, p7), and p6. MA, the N-terminal region of the Gag polyprotein, is important for targeting Gag and Gag-Pol precursor polyproteins to the plasma membrane prior to viral assembly. Furthermore, the MA protein lines the inner surface of the viral membrane, which appears to help incorporate Env glycoproteins into the virion, as well as facilitate infection of non-dividing cell types—mainly macrophages. The CA protein forms the core of the virus particle. Its C-terminal domain functions primarily in assembly, while its N-terminal domain is important for infectivity, through its association with the cellular chaperone cyclophilin A during viral uncoating. The NC protein coats genomic viral RNA and targets it for packaging in the assembling virion; it has also been implicated in other chaperone-like functions through the activity of its two zinc-finger domains, such as melting secondary RNA structures, participating in DNA strand exchange reactions during reverse transcription, and stimulating the integration step. Lastly, p6 is important for incorporating the accessory protein Vpr into the virus during assembly, as well as mediating efficient particle release.

The *pol* region encodes for three proteins, PR (protease, p11), RT (reverse transcriptase), and IN (integrase, p32), which provide essential enzymatic functions. The viral protease is responsible for the final maturation of the otherwise non-infectious viral particles during the assembly and budding stages by cleaving Gag and Gag-Pol polyproteins, allowing the virus to undergo conformational changes. The PR protein (whose protease activity requires

dimerization) is cleaved off from the Gag-Pol polyprotein based on the polyprotein's autocatalytic activity. The viral protease is also responsible for producing the final MA, CA, NC, p6, RT, and IN proteins, making it a prime target for drug design. As a retrovirus, HIV's genomic RNA must first be transcribed into DNA before integration into the host chromosome. The RT enzyme (active as a heterodimer, p66 + p51) is capable of catalyzing both RNA-dependent DNA polymerization (RDDP), also known as reverse transcription, and DNA-dependent DNA polymerization (DDDP), which completes the cDNA strand. Both strands of genomic RNA are needed for this HIV-1 replication, as they form a dimer that acts as the substrate for RT and stabilizes other replication intermediates.[11] The RT enzyme also contains an RNase H domain, which functions to cleave the RNA portion of RNA-DNA hybrids, and also plays a role in creating a maker (a primer on the PPT that is unable to by hydrolyzed by RNase H) that specifies the beginning of the U3 long terminal repeat (LTR). Following reverse transcription, the integrase enzyme catalyzes a reaction that first leaves a 3'-OH CA-dinucleotide overhang on the viral DNA, which later becomes covalently bonded to the 5' ends of the target DNA. The integrase uses negative sense viral DNA as the integration substrate and the host machinery subsequently completes the duplex.

The *env* region codes for the surface (SU, gp120) glycoprotein as well as the viral transmembrane protein (TM, gp41), which dictate the binding, fusion, and tropism characteristics of HIV. The surface glycoprotein acts as a ligand to host CD4 protein, and binds to it, making it the major receptor of HIV-1.

However, HIV must also bind a coreceptor in order to initiate entry. It is thought that the V3 loop of SU (which is exposed upon CD4 binding) determines the viral coreceptor tropism—either CCR5 or CXCR4, although it is likely not the sole determinant given the variability of V3 sequences. Upon binding to CD4, Env (gp160) undergoes structural changes that allow SU to bind to the viral coreceptor and initiate the entry step. Following receptor/coreceptor binding, TM undergoes structural changes and mediates fusion between the viral and cellular membranes.

Beyond its retroviral components, HIV-1 codes for two gene regulatory proteins, Tat and Rev, as well as four accessory proteins, Nef, Vif, Vpr, and Vpu. Tat is essential for viral replication because it increases both the rate of transcriptional initiation and the processivity of the host RNA polymerase II in producing viral proteins. However, unlike most transactivators, instead of binding to DNA, Tat binds to a hairpin structure, TAR, on the nascent viral mRNA, where it is thought to enhance phosphorylation of the C-terminal domain of RNA pol II through an interaction with the general transcription factor TFIIH. Similarly, Rev regulates replication by binding to RRE, a series of RNA hairpin structures that arise during transcription of the *env* region. Rev affects RNA splicing, stability, and contains a nuclear export signal. Rev regulates replication by creating a negative feedback loop that controls nuclear export of unspliced and singly spliced vs. multiply spliced viral mRNA into the cytoplasm. This feedback occurs because most early stage viral mRNAs are multiply spliced and encode the Tat, Rev, and Nef proteins. However, RRE must bind multiple Rev

monomers in order for unspliced and singly spliced viral mRNA to be exported. In this manner, Rev will eventually cause protein production to shift from multiply spliced viral mRNAs to singly spliced or unspliced viral mRNAs, helping to optimize the use of host cellular resources during replication.

The accessory proteins are not necessary for HIV replication, but greatly increase replication efficiency and infectiousness of HIV *in vivo*. For example, Nef helps HIV infected cells escape CTLs through downregulation of MHC I molecules, and escape immune surveillance (e.g. by dendritic cells) through downregulation of CD4 by targeting it to lysosomes. Vif promotes the production of highly infectious mature virions. Its absence is especially noticeable in nonpermissive or semipermissive cell types, where it is thought to mimic a missing host factor utilized during replication. Vif enhances infection by stabilizing DNA replication intermediates and (via an unknown role) functioning in viral assembly and/or maturation. After uncoating, Vpr comes into play by transporting nucleoprotein complexes to nuclear pores (rather than to the interior of the nucleus), where it is theorized to form an ion channel. Additionally, Vpr can induce G2 cell cycle arrest prior to nuclear envelope breakdown and chromosome condensation, thereby increasing infection efficiency in both dividing and nondividing cells. Lastly, the Vpu protein aids in replication at three points. Vpu disentangles Env-CD4 complexes that form in the ER by degrading CD4; Vpu can downregulate MHC I surface expression; and Vpu can stimulate virion release by degrading tetherin, a cellular antiviral protein that inhibits the release of viral particles.[12,24]

Host Immunity

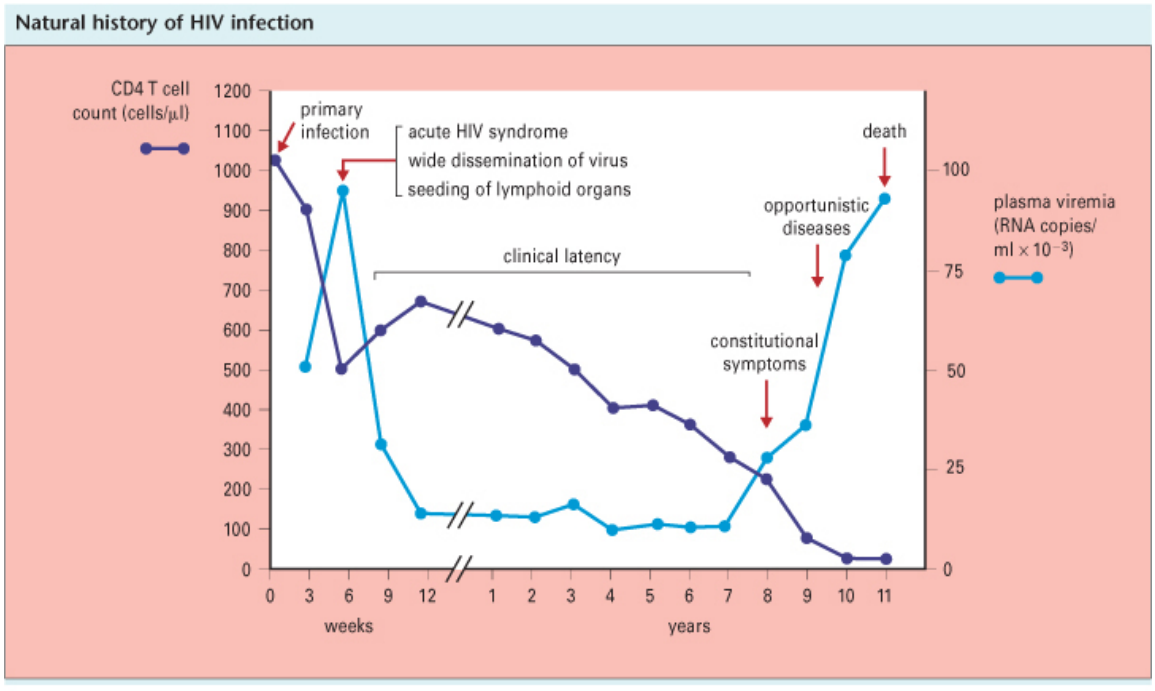
Viruses enter cells by binding to specific proteins expressed on their surface. In the case of HIV-1, the CD4 antigen is the main receptor for viral entry; it is present on the following immune cells: $CD4^+$ T lymphocytes, monocytes, dendritic cells, and brain microglia. Additionally, in cells lacking CD4 receptors, such as fibroblasts, an Fc receptor site or complement receptor site may be used instead for entry of HIV; gp120-independent infection has also been reported.[13,14] The CD4 protein interacts with MHC class II receptors used by immune cells for antigen presentation—a process whereby other immune cells display the degraded fragments of proteins from invading pathogens to T cells. The T cells recognize these antigens via specialized antigen receptors—T Cell Receptors (TCRs)—which share structural similarities to antibodies, however TCRs can only recognize a pathogen's protein fragments when bound to MHC molecules. Upon antigen recognition, $CD4^+$ T cells can then activate other immune cells (including B cells and $CD8^+$ T Cells) to respond to the infection.[15,16]

In addition to the CD4 receptor, HIV-1 must utilize a co-receptor to facilitate entry into the cell; the co-receptor used determines viral tropism. Most primary HIV-1 isolates (new cases of HIV-1 transmission) utilize the CCR5 chemokine receptor (R5 viruses). The other major HIV-1 variant utilizes the CXCR4 chemokine receptor (X4 viruses) as its co-receptor. However, $CD4^+$ T lymphocytes display an unequal distribution between $CCR5^+$ and $CXCR4^+$ cell subtypes; 79% of $CD4^+$ T lymphocytes are exclusively $CXCR4^+$, whereas 4% of

$CD4^+$ T lymphocytes are exclusively $CCR5^+$. Approximately 6% are double positive, and the remaining 11% of $CD4^+$ T cells express neither CCR5 nor CXCR4 at detectable levels on the cell surface. [17]

During the later stages of viral infection, the viral phenotype shifts from R5 to X4 in approximately 50% of individuals.[18] The clinical significance of this switch is that the HIV-1 in circulation has effectively increased its pool of susceptible $CD4^+$ T cells from 10% to 89%.[17] Additionally, CXCR4 is present on several other cell types, including naïve T cells, B cells, and monocytes, which some data have implicated in creating a bystander effect during HIV-1 infection.[19] The increased pool of susceptible cells results in a shift towards rapid T cell infection and depletion, leading to the immune dysfunction characteristic of AIDS patients **(figure 2)**.[20]

Figure 2:[16] *Timeline of HIV-AIDS Progression*



The immune system is unsuccessful in clearing HIV due to three primary viral evasion strategies, namely, latency, mutation/antigenic drift, and subversion of the host immune system. Latency dictates that patients cannot discontinue an effective drug therapy without resurgence of the virus. The mutability of the virus dictates that the emergence of drug resistance is inevitable and that viral load suppression by the host immune system will fade as new HIV quasispecies appear. Meanwhile, HIV interacts with the immune system in such a manner that its activation is an intended outcome in HIV pathogenesis. Given these strategies, it is clear that a multi-pronged and systematic approach to halting HIV pathogenesis is required.[21]

HIV-1 Pathogenesis & LSP1

Since sexual transmission accounts for 75-90% of new HIV infections, HIV-1 transmission through mucosal surfaces has become a growing focal point in the field of HIV research.[4] Dendritic cells are considered one of the early targets of HIV-1 infection and are key players in the wider dissemination of HIV. In what is known as the DC-SIGN pathway, HIV binds to the SIGN molecule with high affinity. However, this binding does not trigger the fusogenic activity needed for productive HIV infection to occur in DCs. Instead, the still-infectious HIV virion can remain bound on the cell's outer surface for many days. Dendritic cells, as antigen presenting cells, inevitably encounter other immune cells. Thus, as the DC forms an immunological synapse with a nearby immune cell, HIV may then bind the necessary receptors to infect the contacted cell.[22]

However, it was discovered that Leukocyte Specific Protein 1 (LSP1, also known as Lymphocyte Specific Protein 1, WP34, Leufactin, 47 KDa Actin Binding Protein, or pp52) interacts with DC-SIGN to mediate transfer of internalized HIV-1-SIGN complexes to the proteasome.[23] This finding was further explored and demonstrated that the absence or downregulation of LSP1, as would be expected, caused enhanced *trans* (cell-to-cell) HIV-1 transmission to T cells.[25] The LSP1 gene encodes for an intracellular filament-actin binding protein, known to bind the ends of actin filaments of the cell membrane together in bundles.[26] Through this activity, LSP1 is able to affect actin polymerization/cytoskeletal remodeling, cellular motility, chemotaxis, and aspects of molecular trafficking.[26,27] Furthermore, LSP1 has a calcium binding domain and is a major downstream substrate of p38 mitogen-activated protein kinase and PKC.[28,29] However, while LSP1 is also expressed in neutrophils, macrophages, activated PBMCs, and endothelial cells, its function in lymphocytes is unknown.[28,30] Since >95% of circulating HIV-1 is produced by newly infected $CD4^+$ T cells, elucidating the role of LSP1 during HIV-1 infection of T cells is key to better understanding HIV-1 pathogenesis.

Materials and Methods

A reverse genetics strategy was employed to study the role of LSP1 during HIV-1 infection in T-cells. To do so, an shRNA plasmid construct was constructed to transcriptionally silence the LSP1 gene. An X4 tropic (T tropic) HIV-1 virus was chosen because of its ability to infect naïve T cells.[32]

Cell Culture

The cell line MT4 was used for this study. MT4 cells were originally derived from an adult with T cell leukemia (HTLV-1 transformed; though rare, low level, transient HTLV shedding is known to occur).[31] X4 HIV-1 strains tend to cause (compared to R5 isolates) syncytium formation and cell death.[32] X4 HIV-1 pathogenesis is especially robust in MT4 cells since in the LTR of HIV-1, the enhancer region of U3 can also bind regulatory proteins of heterologous viruses, in particular, HTLV-1 Tax protein.[49] Once established, the LSP1 knockdown (KD) and non-targeting shRNA (NT) cell lines were cultured in RPMI 1640 medium supplemented with (v/v) 10 % FBS and 1 % Penicillin-Streptomycin (cRPMI). Cells were maintained by removing old media / excess cell growth and replacing the volume with fresh cRPMI (approximately every 2-4 days depending on desired growth and media pH indicator), and incubated at 37 ˚C and 5 % CO_2 in a humidified environment.

Two different (antisense) binding sites on the LSP1 mRNA transcript were (separately) targeted to knockdown LSP1 gene transcripts. This was done using

a stably transfected (Lipofectamine method) shRNA plasmid in MT4 cells; the respective cell lines were dubbed LSP1-09-KD and LSP1-10-KD ("09" and "10"); non-targeting shRNA was used as a control. Following transfection, the 09, 10, and NT constructs were cultured from single cell clones (SCCs). SCCs were established using serial dilutions in a 96 well plate and selected by GFP fluorescence expressed from the plasmid vehicle. Selected SCCs were then subjected to drug selection with Puromycin at a dose of 1 μg/mL (selection dose was optimized; data not shown) for 2 weeks.[33] Drug selection was then discontinued as to not interfere with normal cellular gene or protein expression, since Puromycin is a (quite toxic) protein synthesis inhibitor that functions by causing premature chain release during translation.

Verification of Knockdown Construct

After RNA extraction with Trizol reagent and reverse transcription were performed (per manufacturer's instructions), LSP1 gene expression was quantified by RT-PCR using SYBR® Green manufacturer's protocols.[34] Gene knockdown was periodically monitored throughout the duration of the study (data not shown). Following protein estimation, sample loading was standardized to 50 ng of protein and analyzed by western blot, as described previously.[35] Since the GAPDH gene is often stably and constitutively expressed at high levels in most tissues and cells, and is considered a "housekeeping gene," it was used as a loading control for the western blot.[50]

Cell Proliferation Assay

Since LSP1, through its calcium binding domain, may act in a negative feedback loop in cell cycle control,[29] a CFSE proliferation assay was performed to characterize the cell lines over a 3 day period. CFSE is a non-toxic dye. As labeled parental cells divide, the CFSE fluorescence is partitioned between the daughter cells. Therefore, cell proliferation can be tracked by measuring mean CFSE intensity per cell over time.[36]

Quantification of HIV Production

Effects of LSP1-Knockdown during HIV-1 infection were examined in NT vs. KD cell lines using the X4 tropic HIV-1 strain, HIV_{IIIB}. A quantity of 10^6 cells were incubated with 10 ng p24 of HIV_{IIIB} (~10^7 virion per ng of p24; approximate MOI of 10) for 1 hr at 37 ˚C, and then washed to remove unbound HIV. The supernatant of infected cells was collected days 1-4 post-infection, and the total virion content was quantified by p24 (HIV capsid) ELISA assay.

Examination of HIV Infectivity

After normalizing p24 concentrations from the supernatant of the NT vs. KD cells, the infectiousness of the HIV-1 virion produced was verified by an LTR-driven GHOST reporter cell line, which was quantified by flow cytometry using CellQuest software (BD Biosciences). In this assay, the amount of infectious HIV is measured by proxy of GFP fluorescence, where GFP+ cells indicate HIV infection since GFP is expressed under the control of the viral LTR.[37] Please note: This is not a co-culture experiment; cells expressing GFP

from the plasmid vehicle are not intermixed with the GHOST cell reporter line.

Determination of Replication Interference

In order to determine where LSP1 knockdown may be interfering with viral replication, a viral binding and entry assay was performed as described previously,[35] but are described below briefly. These assays were chosen since the effect of LSP1 knockdown on HIV-1 infection seemed to have been mediated through altered cytoskeletal dynamics given the experimental results which showed discrepancies in both total HIV-1 virion produced and the proportion of infectious HIV-1 virion produced.

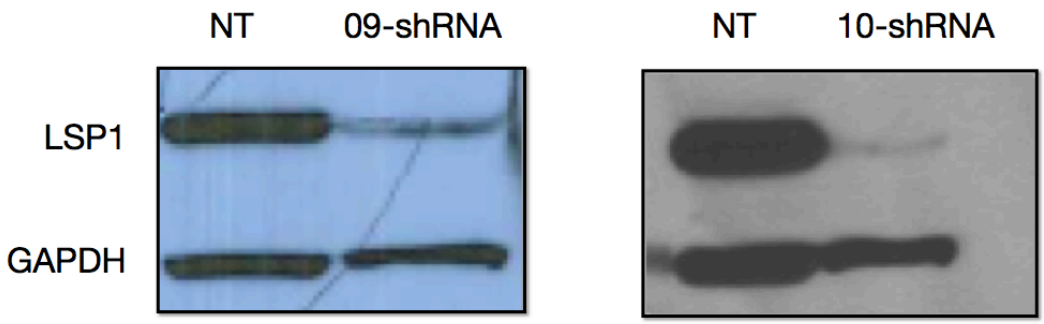
For the virus binding assay, cells were infected with HIV-1_{NL4-3} (X4 tropic) at a concentration of 40 ng p24/10^6 cells. After 1 hr incubation at 4 ˚C, the cells were extensively washed with ice-cold PBS to remove unbound viruses, transferred to fresh tubes, and finally lysed in ice-cold lysis buffer (1% Triton X-100). Total cell protein was estimated, and all samples were normalized for protein content. Virus binding was monitored by measuring the amount of p24 in the cell lysates by ELISA.

For the virus entry assay, cells were incubated with HIV-1_{NL4-3} for 3 hr at 37 ˚C. Following incubation, the cells were washed five times with PBS, treated with trypsin for 5 min at 37 ˚C to remove the uninternalized virus from the surface, and washed once with cRPMI and five times with PBS. Lysis and p24 ELISA were performed in the same manner as during the binding assay.

Results

Before further study could be conducted, the effectiveness of the plasmid construct had to be verified. Western blot analysis confirmed that LSP1 knockdown with the shRNA plasmid construct was effective at reducing LSP1 protein expression **(figure 3)**, without affecting normal gene expression (GAPDH was used as a representative measure).

Figure 3: *Western Blot in transfected MT4 Cells*

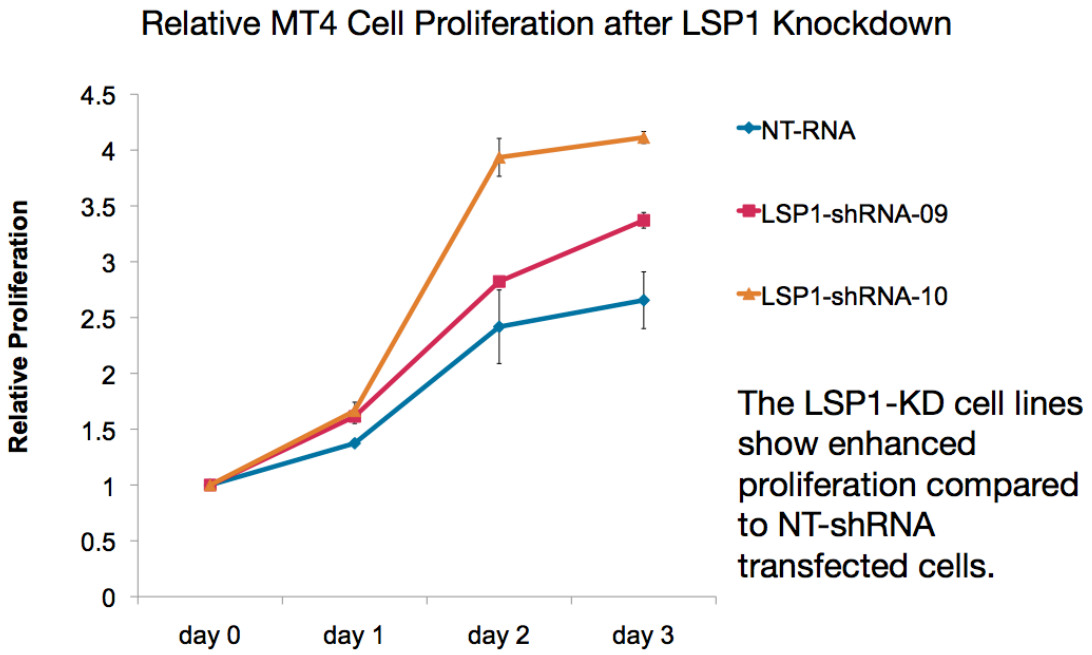


Western Blot analysis confirms that LSP1 expression was effectively knocked down in the transfected MT4 cell lines generated for this study ("LSP1-09-KD" and "LSP1-10-KD").

Cell Proliferation Assay

Thereafter, the cells were characterized for proliferation **(figure 4)**, since as mentioned earlier, HIV-1 accessory protein Vpr induces growth cycle arrest in order to enhance replication efficiency, making cell cycle characteristics an area of interest.

Figure 4: *Viability of Transfected MT4 cells*

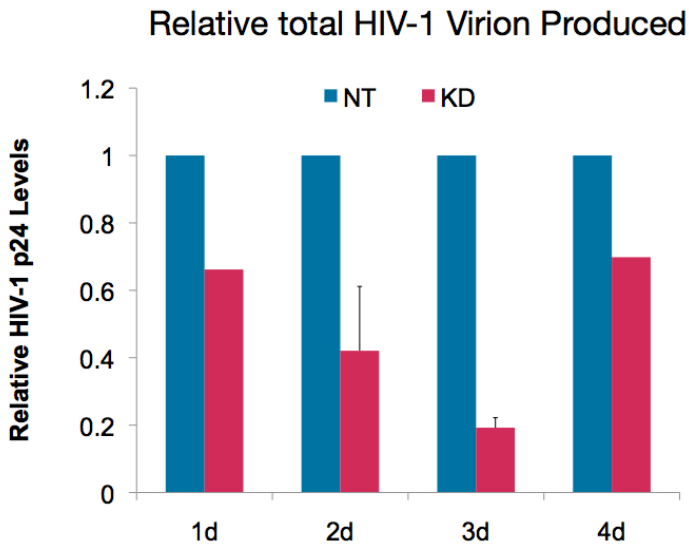


The proliferation assay revealed that LSP1-KD results in enhanced viability, with both the 10-shRNA and 09-shRNA variants displaying an increase in proliferation (fig. 4). This data also gives credence to a previous hypothesis that knockdown of LSP1 would accelerate the cellular growth cycle by eliminating LSP1 from a negative feedback loop involving calcium regulation.[29] Additionally, based on a qualitative assessment of the western blot results, LSP1 knockdown appears to accelerate the growth cycle / cell proliferation in a dose dependent manner since the relative proliferation, 10 > 09 > NT, is concordant with the relative protein expression shown in the western blot. As such, a future area of study would be to examine the cell cycles of LSP1-KD vs. NT cells during infection with WT HIV-1 or an HIV-1 Vpr-mutant.

Quantification of HIV Production

The ability of the LSP1-KD cell line was then assessed for its ability to support productive HIV-1 infection. The total amount of virion produced was measured by p24 ELISA **(figure 5)**.

Figure 5: *Synchronized HIV-1 Infection of MT4 cells*



Cells were incubated with HIV-1 for 1 hr, and then washed to remove unbound HIV.

The supernatant of the LSP1-KD cells showed less replication of HIV-1 as detected by p24 ELISA.

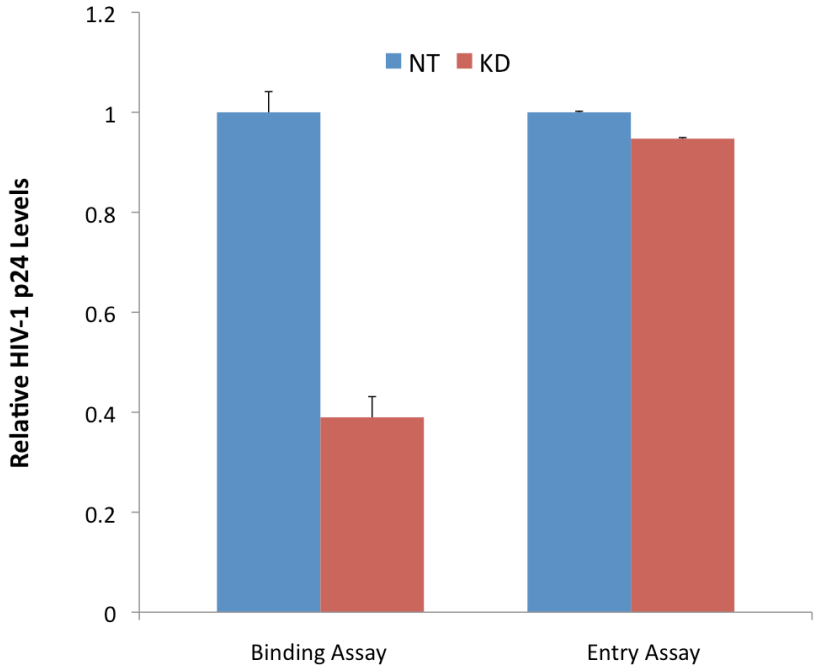
The data (fig. 5) indicates a substantial and sustained drop in newly produced HIV-1 p24 levels, with 40%, 60%, and 80% reductions in relative virion production over days 1-3. The increase in relative p24 levels at day 4 may at first seem to indicate decay in the knockdown's protective effect. However, a cursory examination under a microscope revealed that this effect was likely due to the cytopathic effects of HIV-1 infection. That is—nearly all the NT cells had died and therefore generated no additional virion. Meanwhile, since viruses

replicate in bursts, and HIV-1 on average requires 52 hr between the export of one generation of virions and the next, the increase in relative p24 levels on day 4 in LSP1-KD cells is most likely the 2nd replication burst.[38,39]

Determination of Replication Interference

Since the earlier quantification of relative total p24 levels indicated that HIV replication is being inhibited when LSP1 is knocked down, it is important to determine where in the viral life cycle this is occurring as to narrow down what interaction is mediating the inhibition of replication. Binding and entry were analyzed as being among the more likely interference points **(figure 6)**.

Figure 6: *Examination of HIV-1 Binding and Entry with LSP1-KD in MT4 cells*

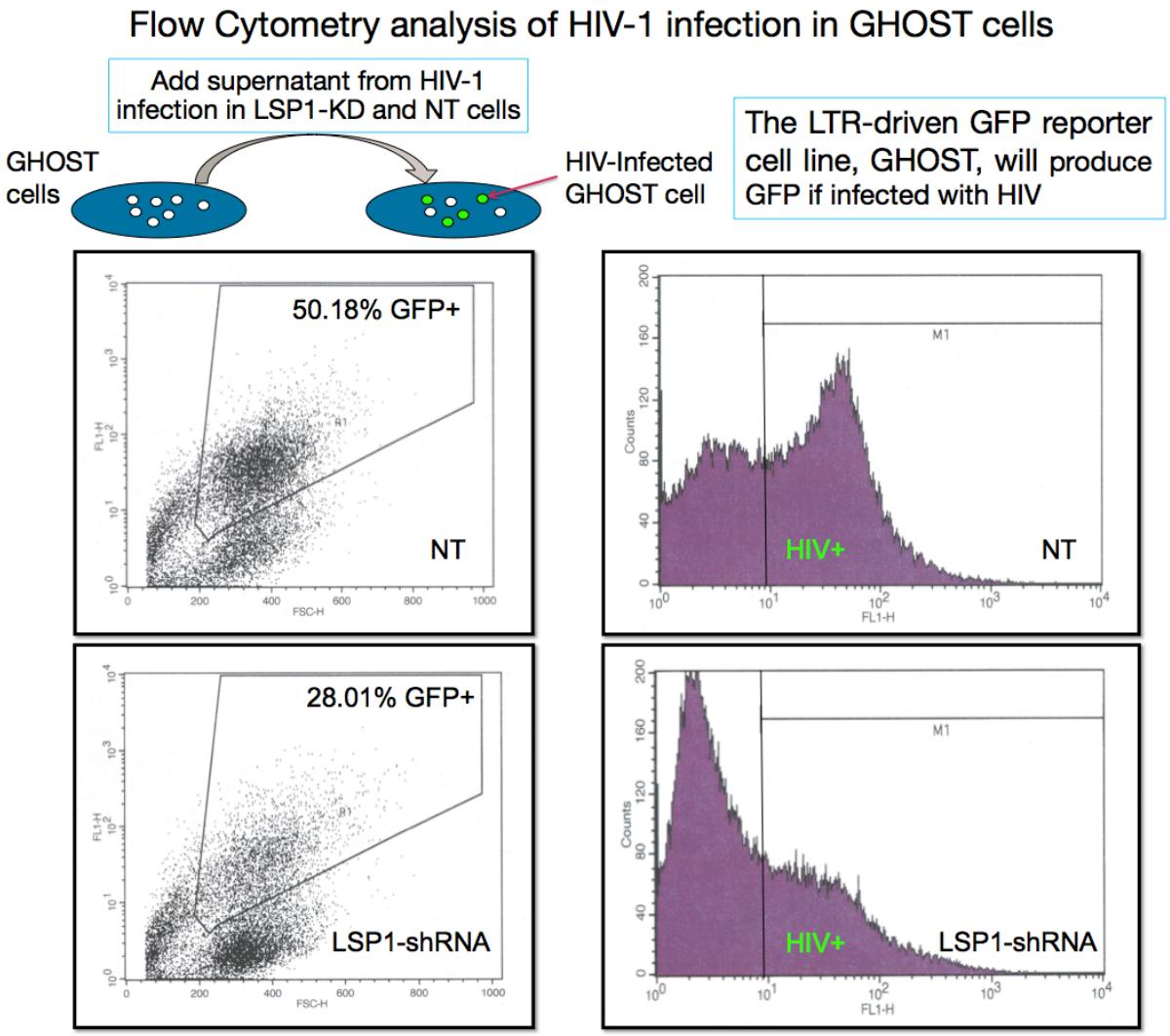


The binding assay (fig. 6) showed a significant reduction ($p = 0.02$) in HIV-1 binding in LSP1-KD cells—62%, while the entry assay showed no significant differences. The significance and implications of these results will be explored in the discussion section.

Examination of HIV Infectivity

However, a measurement of total virion produced alone may not illustrate the full protective effect of LSP1 knockdown against HIV-1 infection. In retroviruses such as HIV, the ratio of noninfectious to infectious physical particles typically ranges from 10^5:1 to 10^7:1.[39] As such, it was prudent to examine if the HIV-1 particles that were produced during infection of LSP1-KD cells were as infectious as those of the control sample. Therefore, the relative amount of infectious virion produced was compared **(figure 7)** using a reporter cell line, GHOST cells, wherein GFP is expressed under the control of an LTR, which is activated upon infection with a retrovirus.

Figure 7: *Infection of GHOST cells using infected LSP1-KD and NT Supernatant*



Note: Greater GFP fluorescence indicates more HIV infection. (FL1-H = GFP Intensity) Cells within the region labeled M1 (displayed right) are considered to be infected.

The data (fig. 7) indicates that LSP1-KD cells produce a smaller proportion of *infectious* HIV-1 virion (vs. total HIV-1 virion) compared to NT controls. Stated otherwise, the relative infectiousness of HIV-1 in the supernatant from infection of LSP1-KD cells vs. NT cells was reduced by 44% with LSP1 knockdown.

Discussion

HIV-1 infections have proved extremely difficult to overcome through conventional interventions because of the mutability and evasiveness of the virus. As such, a growing body of evidence supports a case for the notion that therapies directed towards human endogenous targets rather than viral targets may be a more successful way to control HIV-1 infections.[7,8,9] Previous studies of LSP1 made a convincing argument that LSP1 knockdown would enhance HIV-1 spread through cell-to-cell transmission (from DCs to T cells) because LSP1 binds to the cytoplasmic region of DC-SIGN (independently of LSP1's F-actin binding domain) and helps to direct DC-SIGN bound to HIV-1 to the proteasome.[23] However, the above data taken in context with previous work form the Ganju lab and others[48] seems to indicate that a more nuanced effect would arise *in vivo*.

Previously,[28] we have shown that stimulation with gp120 induced iDC chemotaxis via a downstream signaling pathway **(figure 8)** that activates LSP1.

Figure 8: *Proposed Signaling Pathway for gp120-induced chemotaxis of iDCs*

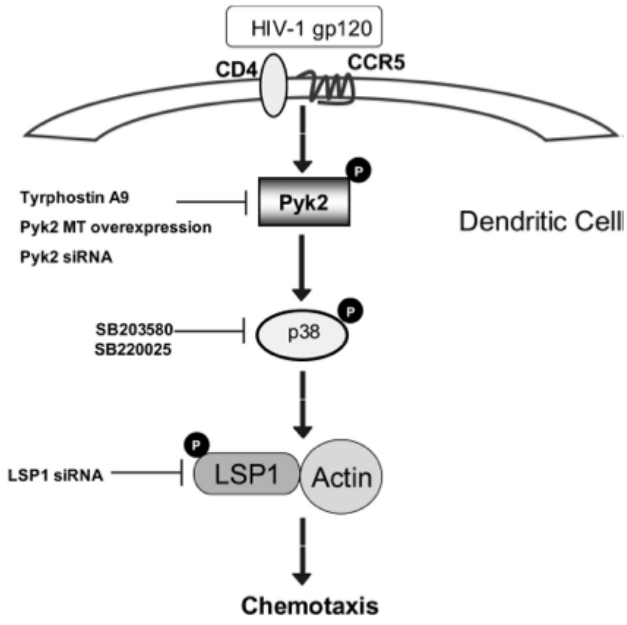


Image from Ganju, et al., 2009.[28]

In that study, an siRNA knockdown of LSP1 was utilized, which is a less efficient (only ~75% downregulation) and more transient knockdown than the shRNA construct used in this study, resulted in a 53% attenuation in gp120 induced iDC chemotaxis because LSP1's association with F-actin is need to induce chemotaxis. A stronger LSP1 knockdown would likely amplify this attenuation. Thus, by inhibiting gp120 induced APC migration, viral spread might be lessened.

However, to conclude that the importance of LSP1 is limited to its association with F-actin (which seems to be the extent of most publication's background information on it) would neglect some of the earliest findings about

LSP1. Three distinct subcellular pools of LSP1 protein are known to exist—cytoplasmic, cytoskeletal, and plasma membrane associated pools.[40] This implicates distinct functional significance and differing post-translational modifications. The cytoplasmic LSP1 (which also translocates to the cytoskeletal pool upon phosphorylation by PKC) likely functions as an intermediate effector molecule by responding to the transient Ca^{2+} influxes that occur when membrane receptor cross-linking occurs.[40] The LSP1 Ca^{2+} binding domain is evolutionarily conserved across species, which strongly suggests a continuing functional importance.[40] The cytoskeletally associated LSP1 is known to be phosphorylated by casein kinase II, protein kinase C and MAPKAPK2.[41] The plasma membrane associated LSP1 is N-glycosylated and capable of interacting with immunoglobin-like domains.[42] Several isoforms of LSP1 exist,[30] so the N-glycosylated variant is all but certainly the result of a yet to be indentified alternative splicing combination.

Confocal microscopy data from previous studies in our lab support findings observed in early LSP1 studies regarding B cell activation about its participation in a capping/polarization effect when an Ig-like receptor binds a ligand. The authors theorized that LSP1 capping was occurring either due to Ca^{2+} related signaling events causing cytoplasmic LSP1 translocation, or, due to LSP1 association with the cytoskeleton.[40] While a non-specific effect from Ca^{2+} related signaling events is difficult to rule out,[46,47] our imaging data[28] **(figure 9, left)** supports the LSP1-cytoskeleton "capping" hypothesis through an effect on

actin. This is further confirmed through the use of a drug inhibitor of LSP1 phosphorylation by p38 **(figure 9, right)**.

Figure 9: *Confocal Microscopy of gp120-incuced Cytoskeleton Rearrangements*

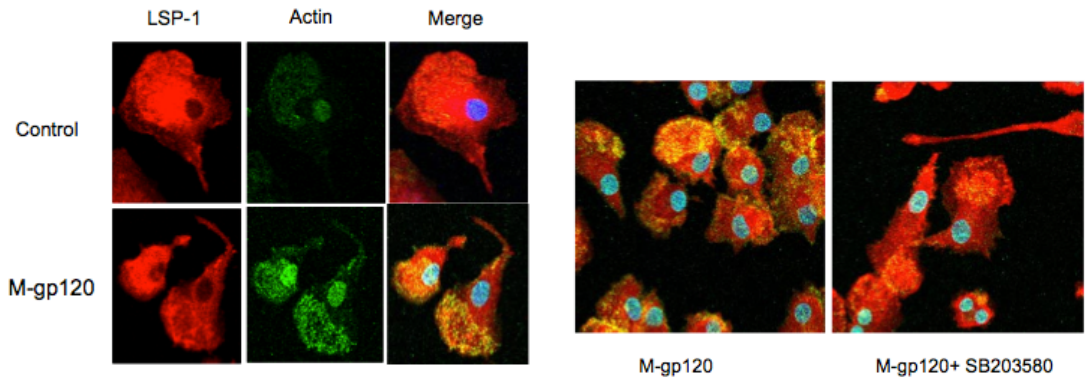


Image from Ganju, et al., 2009.[28]

With this data in mind, now consider the results of the HIV-1 binding and entry assay in the LSP1-KD vs. NT cells. HIV-1 binding was significantly inhibited in LSP1-KD cells compared to NT, while virtually no difference in the amount of internalized p24 was observed in the entry assay. It remains unclear why HIV-1 binding was inhibited in LSP1-KD cells, however, detailed studies on the HIV-1 binding step strongly contradict explanations involving Ca^{2+}, binding kinetics, receptor signaling, and the presence or absence of other GPCR-related signaling proteins.[43,44,45] The only explanation that is consistent with this data is that LSP1-KD (through an unknown mechanism) reduces the binding affinity of CD4 to gp120. It is consistent because it has been shown that high affinity CD4-gp120 binding is not necessary for HIV-1 entry because the binding step is fully

reversible.[43] Meanwhile, the fusion step is rapid enough that making initial contact with the co-receptor is essentially irreversible.[44] As such, the experimenters noted that relatively normal levels of HIV-1 entry had been observed even in instances where no binding was detected by the assay.[44]

Therefore, given the remaining data (the decrease in the relative *total* HIV-1 virion produced and the decrease in the relative proportion of *infectious* HIV-1 virion produced), a reasonable inference can be made about where in the replication cycle the LSP1 knockdown is creating interference. The reduced proportion of infectious virion points toward an effect at the maturation stage (post-egress). Alternatively, this could be occurring due to an interaction with LSP1 and the Nef accessory protein, which is probable based on previous data from our lab which showed a close co-localization of Nef and F-actin **(figure 10)**.[35] Since Nef is packaged in the virion and increases the infectiousness of the virus, it is feasible that the reduced infectiousness of the LSP1-KD cells is mediated through interference with Nef packaging.

Figure 10: *Co-Localization of Virus & Host Factors during HIV-1 Infection*

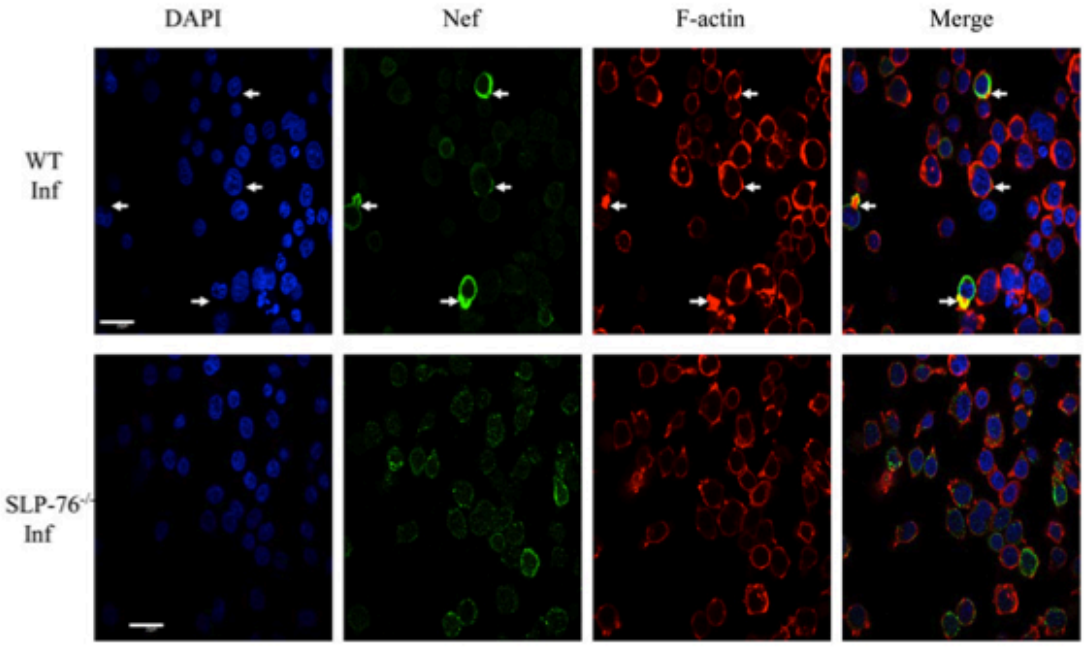


Image from Ganju, et al., 2012.[35]

In contrast, the reduced total HIV-1 virion content could point to an effect during the budding/egress stage, in which case the HIV-1 virion would be retained intracellularly, similar what occurs when tetherin protein expression is upregulated.[12]

Conclusion

Given the multitude of possible functional interactions LSP1 is capable of participating in, this study was neither meant to resolve—nor capable of resolving—the nature of the interaction that is occurring with LSP1 during HIV-1 infection. It is, however, able to provide clues, as well as tantalizing data about the functional outcomes that might be achieved from further study.

Overall, this study does yield the interesting finding that LSP1-KD cells showed lower levels of HIV infection despite enhanced cell viability, meaning that less infection occurred despite there being more available cells to infect. Additionally, it demonstrated that LSP1-KD reduces total HIV-1 replication as well as decrease the infectiousness of the HIV-1 virion produced. Since permissive HIV-1 infection in $CD4^+$ T cells is the primary contributor to viral load in HIV+ persons, therapeutic strategies that target LSP1 could prove promising for patients whose viremia is not well controlled or patients who present with severe $CD4^+$ T cell depletion. Therefore, understanding the physiology of LSP1 during HIV-1 pathogenesis could provide novel therapeutic strategies to protect against HIV-1 and enhance T-cell viability.

References

1.) A Timeline of AIDS. (n.d.). Retrieved April, 2014, from http://aids.gov/hiv-aids-basics/hiv-aids-101/aids-timeline/

2.) AIDS is Officially Recognized. (n.d.). Retrieved April, 2014, from http://www.pbs.org.proxy.lib.ohio-state.edu/wgbh/aso/databank/entries/dm81ai.html

3.) WHO, USAID, & Kaiser Family Foundation. (2012, June 06). THE GLOBAL HIV/AIDS CRISIS TODAY. Retrieved March 30, 2014, from http://aids.gov/hiv-aids-basics/hiv-aids-101/global-statistics/

4.) Cohen, MS. Preventing Sexual Transmission of HIV. Clinical Infectious Disease. (2007) 45 (Supplement 4): S287-S292 doi:10.1086/522552. Retrieved April, 2014, from http://cid.oxfordjournals.org/content/45/Supplement_4/S287.full

5.) AIDS Foundation of Chicago. (n.d.). HIV/AIDS Facts & Figures in the United States. Retrieved March 30, 2014, from http://events.aidschicago.org/site/PageServer?pagename=t2_facts_and_figures

6.) Schackman BR, Gebo KA, Walensky RP, et al. The lifetime cost of current human immunodeficiency virus care in the United States. Med Care. 2006;44:990–7. doi: 10.1097/01.mlr.0000228021.89490.2a. Retrieved April, 2014, from http://www.ncbi.nlm.nih.gov/pubmed/17063130

7.) DiGiusto DL, Stan R, Krishnan A, et al. Development of hematopoietic stem cell based gene therapy for HIV-1 infection: considerations for proof of concept studies and translation to standard medical practice. Viruses. 2013;5:2898–919. Retrieved April, 2014, from

8.) Burnett J.C., Zaia J.A., Rossi J.J. Creating genetic resistance to HIV (Review) Curr. Opin. Immunol. 2012;24:625–632. doi: 10.1016/j.coi.2012.08.013. Retrieved April, 2014, from http://www.ncbi.nlm.nih.gov/pmc/articles/PMC3478429/

9.) Rodicia S, Zaia JA. Practical Considerations in Gene Therapy for HIV Cure. Current HIV/AIDS Reports. 2014; 11: 11–19. Published Jan 22,

2014. doi: 10.1007/s11904-013-0197-1. Retrieved April, 2014, from http://www.ncbi.nlm.nih.gov/pmc/articles/PMC3929767/

10.) Frankel A, Young J. 1998. HIV-1: fifteen proteins and an RNA. Annual Review of Biochemistry 67:1–25.10.1146/annurev.biochem.67.1.1. Retrieved April, 2014, from http://www.annualreviews.org/doi/abs/10.1146/annurev.biochem.67.1.1

11.) Moore, M. D., & Hu, W. (2011, March 14). HIV-1 RNA Dimerization: It Takes Two to Tango. Retrieved April, 2014, from http://www-ncbi-nlm-nih-gov.proxy.lib.ohio-state.edu/pmc/articles/PMC3056336/

12.) Mangeat, B., Gers-Huber, G., Lehmann, M., Zufferey, M., Luban, J., & Piguet, V. (2009, September 04). HIV-1 Vpu Neutrawuzes the Antiviral Factor Tetherin/BST-2 by Binding It and Directing Its Beta-TrCP2-Dependent Degradation. DOI: 10.1371/journal.ppat.1000574. Retrieved March/April, 2014, from http://www.plospathogens.org.proxy.lib.ohio-state.edu/article/info%253Adoi%252F10.1371%252Fjournal.ppat.1000574

13.) Stoiber H. 2009. Complement, Fc receptors and antibodies: a Trojan horse in HIV infection? Curr. Opin. HIV AIDS 4:394–399. doi: 10.1097/COH.0b013e32832f0108.

14.) Chow YH, Yu D, Zhang JY, Xie Y, Wei OL, et al. gp120-Independent infection of CD4(–) epithelial cells and CD4(+) T-cells by HIV-1. J Acquir Immune Defic Syndr. 2002;30:1–8. Retrieved April, 2014, from http://journals.lww.com/jaids/Abstract/2002/05010/gpl20_Independent_Infection_of_CD4__Epithelial.1.aspx

15.) Robinson JH, Delvig AA. Diversity in MHC class II antigen presentation. Immunology. 2002;105:252–262. Retrieved April, 2014, from http://www.ncbi.nlm.nih.gov/pmc/articles/PMC1782669/

16.) Male, David. *Immunology*. 7th ed. Philadelphia, PA: Mosby Elsevier, 2006. Print. PP. 37, 105-106, 316-320, 509.

17.) Grivel J.-C., Margolis L. B. (1999) CCR5- and CXCR4-tropic HIV-1 are equally cytopathic for their T-cell targets in human lymphoid tissue. Nat. Med. 5:344–346. Retrieved April, 2014, from http://www.nature.com/nm/journal/v5/n3/full/nm0399_344.html

18.) Connor, R. I., K. E. Sheridan, D. Ceradini, S. Choe, and N. R. Landau. 1997. Change in coreceptor use correlates with disease progression in HIV-1-infected individuals. J. Exp. Med. 185:621-628. Retrieved April, 2014, from http://jem.rupress.org/content/185/4/621.abstract?ijkey=bf8adfe86f283deafa7c45eccccc6804a7fe31d2&keytype2=tf_ipsecsha

19.) T.H. Finkel, G. Tudor-Williams, N.K. Banda, M.F. Cotton, T. Curiel, C. Monks, T.W. Baba, R.M. Ruprecht, A. Kupfer. Apoptosis occurs predominantly in bystander cells and not in productively infected cells of HIV- and SIV-infected lymph nodes. Nat. Med., 1 (1995), pp. 129–134. Retrieved April, 2014, from http://www.nature.com.proxy.lib.ohio-state.edu/nm/journal/v1/n2/full/nm0295-129.html

20.) Grivel JC, Penn ML, Eckstein DA, Schramm B, Speck RF, et al. (2000) Human immunodeficiency virus type 1 coreceptor preferences determine target T-cell depletion and cellular tropism in human lymphoid tissue. J Virol 74: 5347–5351. Retrieved April, 2014, from http://jvi.asm.org/content/74/11/5347.full

21.) Debjani Guha and Velpandi Ayyavoo, “Innate Immune Evasion Strategies by Human Immunodeficiency Virus Type 1,” ISRN AIDS, vol. 2013, Article ID 954806, 10 pages, 2013. doi:10.1155/2013/954806. Retrieved April, 2014, from http://www.hindawi.com/journals/isrn.aids/2013/954806/

22.) Wu L. Biology of HIV mucosal transmission. Current Opinion in HIV and AIDS. 2008;3(5):534–540. Retrieved April, 2014, from http://www.ncbi.nlm.nih.gov/pmc/articles/PMC2542876/

23.) Smith AL, Ganesh L, Leung K, Jongstra-Bilen J, Jongstra J, Nabel GJ. Leukocyte-specific protein 1 interacts with DC-SIGN and mediates transport of HIV to the proteasome in dendritic cells. *Journal of Experimental Medicine*. 2007;204(2):421–430. Retrieved April, 2014, from http://www.ncbi.nlm.nih.gov/pmc/articles/PMC2118718/

24.) Schubert U, Anton LC, Bacik I, Cox JH, Bour S, Bennink JR, Orlowski M, Strebel K, Yewdell JW. CD4 glycoprotein degradation induced by human immunodeficiency virus type 1 Vpu protein requires the function of proteasomes and the ubiquitin-conjugating pathway. J Virol. 1998;72:2280–2288. Retrieved April, 2014, from http://www.ncbi.nlm.nih.gov/pmc/articles/PMC109526/

25.) Charles R. Rinaldo, “HIV-1 Trans Infection of CD4+ T Cells by Professional Antigen Presenting Cells,” Scientifica, vol. 2013, Article ID 164203, 2013. doi:10.1155/2013/164203. Retrieved April, 2014, from http://www.ncbi.nlm.nih.gov/pmc/articles/PMC3820354/

26.) Jongstra-Bilen J, Janmey PA, Hartwig JH, Galea S, Jongstra J. The lymphocyte-specific protein LSP1 binds to F-actin and to the cytoskeleton through its COOH-terminal basic domain. J Cell Biol. 1992;118:1443–1453. Retrieved April, 2014, from http://www.ncbi.nlm.nih.gov/pmc/articles/PMC2289610/

27.) Kadiyala, R. K., McIntyre, B. W., & Krensky, A. M. (1990). Molecular cloning and characterization of WP34, a phosphorylated human lymphocyte differentiation and activation antigen. Retrieved April, 2014, from http://www-ncbi-nlm-nih-gov.proxy.lib.ohio-state.edu/pubmed/2174784

28.) Anand AR, Prasad A, Bradley RR, Deol YS, Nagaraja T, Ren X, Terwilliger EF, Ganju RK. HIV-1 gp120-induced migration of dendritic cells is regulated by a novel kinase cascade involving Pyk2, p38 MAP kinase, and LSP1. Blood. 2009;114:3588–3600. Retrieved April, 2014, from http://www.ncbi.nlm.nih.gov/pmc/articles/PMC2766677/

29.) Klein, D. P., J. Jongstra-Bilen, K. Ogryzlo, R. Chong, and J. Jongstra. 1989. Lymphocyte-specific Ca2+-binding protein LSP1 is associated with the cytoplasmic face of the plasma membrane. Mol. Cell. Biol. 9:3043-3048. Retrieved April, 2014, from http://www.ncbi.nlm.nih.gov/pmc/articles/PMC362773/

30.) Genes and mapped phenotypes - LSP1 lymphocyte-specific protein 1 [*Homo sapiens* (human)]. (n.d.). Retrieved April, 2014, from http://www-ncbi-nlm-nih-gov.proxy.lib.ohio-state.edu/gene?Db=gene&Cmd=ShowDetailView&TermToSearch=4046

31.) Reagent Datasheet Detail: Catalog 120 - MT-4 - NIH AIDS Reagent Program. (n.d.). Retrieved April, 2014, from https://www.aidsreagent.org/reagentdetail.cfm?t=cell_lines&id=352

32.) Fouchier R. A. M., Meyaard L., Brouwer M., Hovenkamp E., Schuitemaker H. (1996) Broader tropism and higher cytopathicity for CD4+ T cells of a syncytium-inducing compared to a non-syncytium-inducing HIV-1 isolate as a mechanism for accelerated CD4+ T cell

decline in vivo. Virology 219:87–95. Retrieved April, 2014, from http://jvi.asm.org/content/75/21/10455.full#ref-16

33.) Optimization of Antibiotic Selection Using a Cytotoxicity Profile. (n.d.). Retrieved April, 2014, from http://www.sigmaaldrich.com.proxy.lib.ohio-state.edu/life-science/functional-genomics-and-rnai/shrna/learning-center/cytotoxicity-profile.html

34.) Universal SYBR Green Quantitative PCR Protocol. (n.d.). Retrieved from http://www.sigmaaldrich.com.proxy.lib.ohio-state.edu/technical-documents/protocols/biology/sybr-green-qpcr.htm

35.) Nagaraja T, Anand AR, Zhao H, Ganju RK. The adaptor protein SLP-76 regulates HIV-1 release and cell-to-cell transmission in T cells. J Immunol. 2012;10:2769–2777. doi: 10.4049/jimmunol.1102106. Retrieved April, 2014, from http://www.ncbi.nlm.nih.gov/pmc/articles/PMC3908818/

36.) Parish CR. Fluorescence for lymphocyte migration and proliferation studies. Immunol Cell Biol 1999, 77: 499–508. Retrieved April, 2014, from http://www.nature.com/icb/journal/v77/n6/full/icb199966a.html

37.) Vödrös D, Tscherning-Casper C, Navea L, Schols D, De Clercq E, Fenyo EM. Quantitative evaluation of HIV-1 coreceptor use in the GHOST3 cell assay. Virology. 2001;291(1):1–11. Retrieved April, 2014, from http://www.ncbi.nlm.nih.gov/pubmed/11878871

38.) Murray J. M., Kelleher A. D., Cooper D. A., 2011 Timing of the components of the HIV life cycle in productively infected CD4+ T cells in a population of HIV-infected individuals. *J. Virol.* 85: 10798–10805. Retrieved April, 2014, from http://jvi.asm.org/content/85/20/10798.full

39.) John L. Spouge and Scott P. Layne. A practical method for simultaneously determining the effective burst sizes and cycle times of viruses. PNAS 1999 96 (12) 7017-7022; doi:10.1073/pnas.96.12.7017. Retrieved April, 2014, from http://www.ncbi.nlm.nih.gov/pmc/articles/PMC22041/

40.) Klein, D.P., S. Galea, and J. Jongstra. 1990. The lymphocyte-specific protein LSP1 is associated with the cytoskeleton and co-caps with membrane IgM. J. Immunol. 145:2967–2973. Retrieved April, 2014, from http://deepblue.lib.umich.edu/handle/2027.42/40391

41.) LSP1 Gene. (n.d.). Retrieved April, 2014, from http://www.genecards.org/cgi-bin/carddisp.pl?gene=LSP1

42.) Patent US20030082677 - Novel EDIRF, MTR-1, LSP-1, TAP-1, and PA-I molecules and uses therefor - Google Patents. (n.d.). Retrieved April, 2014, from http://www.google.com.proxy.lib.ohio-state.edu/patents/US20030082677

43.) Doranz BJ, Baik SS, Doms RW. Use of a gp120 binding assay to dissect the requirements and kinetics of human immunodeficiency virus fusion events. J Virol. 1999;73(12):10346–10358. Retrieved April, 2014, from http://www.ncbi.nlm.nih.gov/pmc/articles/PMC113090/

44.) Baik, S. S., R. W. Doms, and B. J. Doranz. 1999. HIV and SIV gp120 binding does not predict coreceptor function. Virology 259:267-273. Retrieved April, 2014, from http://www.sciencedirect.com/science/article/pii/S0042682299997799

45.) Del Corno M, Liu QH, Schols D, de Clercq E, Gessani S, Freedman BD, et al. HIV-1 gp120 and chemokine activation of Pyk2 and mitogen-activated protein kinases in primary macrophages mediated by calcium-dependent, pertussis toxin-insensitive chemokine receptor signaling. Blood. 2001;98:2909–2916. Retrieved April, 2014, from http://www.ncbi.nlm.nih.gov/pubmed/11698270

46.) Zhou Y., Frey T.K., Yang J.J. Viral calciomics: interplays between Ca2+ and virus. Cell Calcium. 2009;46:1–17. Retrieved April, 2014, from http://www.ncbi.nlm.nih.gov/pmc/articles/PMC3449087/

47.) Ebenbichler CF, Stoiber H, Schneider R, Patsch JR, Dierich MP. The human immunodeficiency virus type 1 transmembrane gp41 protein is a calcium-binding protein and interacts with the putative second-receptor molecules in a calcium-dependent manner. J Virol. 1996;70:1723–1728. Retrieved April, 2014, from http://www.ncbi.nlm.nih.gov/pmc/articles/PMC189996/

48.) Tsunetsugu-Yokota, Y., & Muhsen, M. (2013, July 09). Development of human dendritic cells and their role in HIV infection: Antiviral immunity versus HIV transmission. D38O38I38=38138038.38338338838938/38f38m38i38c38b38.38238038138338.38038038138738838. Retrieved April, 2014, from http://journal.frontiersin.org/Journal/10.3389/fmicb.2013.00178/full

49.) Bohnlein E., Siekevitz M., Ballard D. W., Lowenthal J. W., Rimsky L., Bogerd H., et al. (1989). Stimulation of the human immunodeficiency virus type 1 enhancer by the human T-cell leukemia virus type I tax gene product involves the action of inducible cellular proteins. *J. Virol.* 63 1578–1586. Retrieved April, 2014, from http://www.ncbi.nlm.nih.gov/pmc/articles/PMC248395/

50.) Barber RD, Harmer DW, Coleman RA, Clark BJ (May 2005). "GAPDH as a housekeeping gene: analysis of GAPDH mRNA expression in a panel of 72 human tissues". Physiol. Genomics 21 (3): 389–95. doi:10.1152/physiolgenomics.00025.2005. Retrieved April, 2014, from http://www.ncbi.nlm.nih.gov/pubmed/15769908

Further Reading

51.) Prasad A, Kuzontkoski PM, Shrivastava A, Zhu W, Li DY, et al. (2012) Slit2N/Robo1 Inhibit HIV-gp120-Induced Migration and Podosome Formation in Immature Dendritic Cells by Sequestering LSP1 and WASp. PLoS ONE 7(10): e48854. doi:10.1371/journal.pone.0048854. Retrieved April, 2014, from http://www.plosone.org/article/info%3Adoi%2F10.1371%2Fjournal.pone.0048854

52.) Ghanam R. H., Fernandez T. F., Fledderman E. L., Saad J. S. (2010) Binding of calmodulin to the HIV-1 matrix protein triggers myristate exposure. J. Biol. Chem. 285, 41911–41920. Retrieved April, 2014, from http://www.jbc.org/content/285/53/41911.full

53.) Selliah, N., T. H. Finkel. 2001. HIV-1 NL4-3, but not IIIB, inhibits JAK3/STAT5 activation in CD4(+) T cells. *Virology* 286: 412–421. Retrieved April, 2014, from http://www.sciencedirect.com/science/article/pii/S0042682201909948

54.) Nikolic DS, Lehmann M, Felts R, et al. HIV-1 activates Cdc42 and induces membrane extensions in immature dendritic cells to facilitate cell-to-cell virus propagation. *Blood*. 2011;118(18):4841–4852. Retrieved April, 2014, from http://www.ncbi.nlm.nih.gov/pmc/articles/PMC3208294/

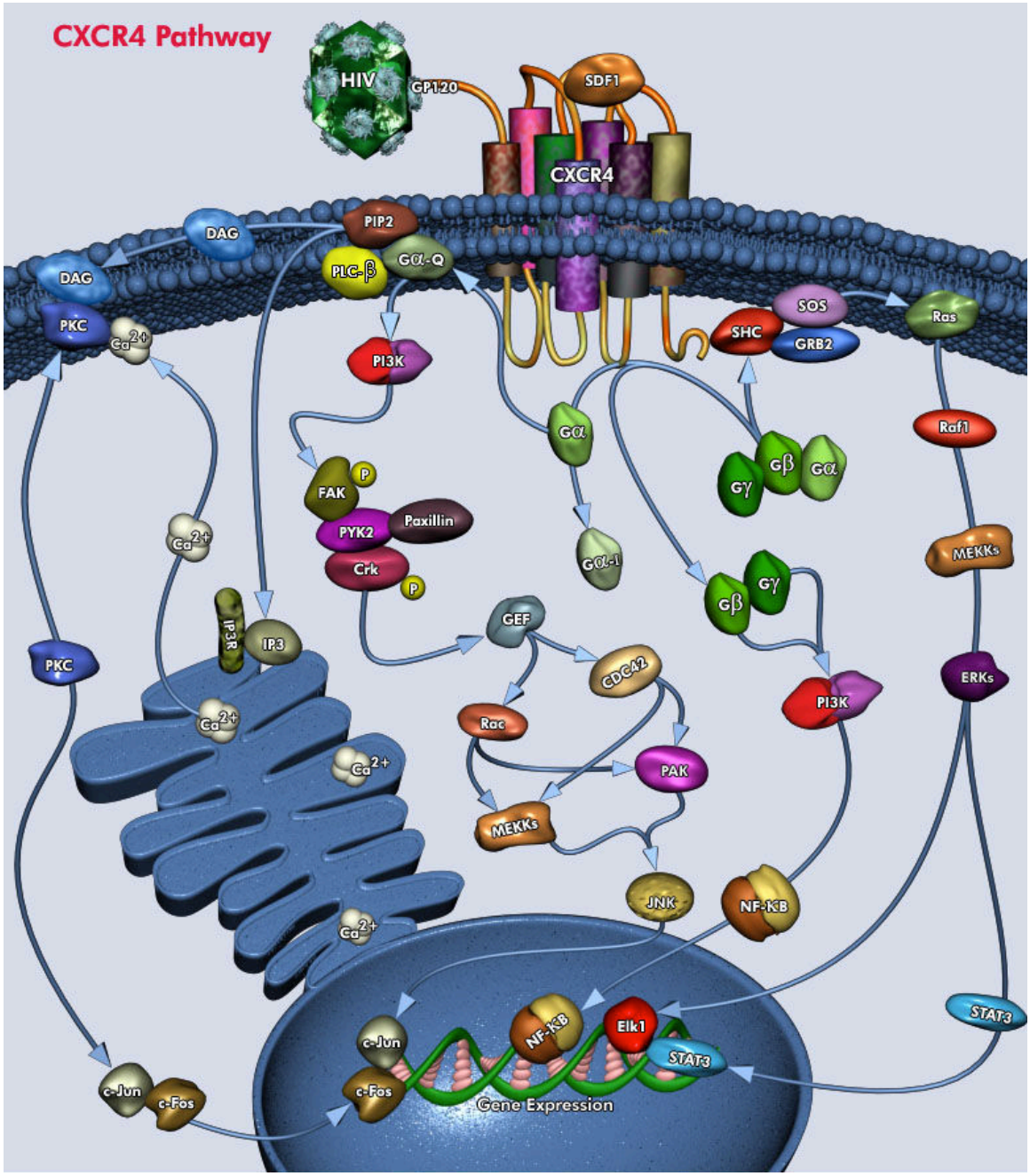
55.) Briz V, Poveda E, Soriano V (2006) HIV entry inhibitors: mechanisms of action and resistance pathways. Journal of Antimicrobial Chemotherapy 57: 619–627. Retrieved April, 2014, from http://jac.oxfordjournals.org/content/57/4/619.full.pdf?origin=publication_detail

56.) Detre C., Keszei M., Romero X., Tsokos G. C., Terhorst C. (2010) SLAM family receptors and the SLAM-associated protein (SAP) modulate T cell functions. Semin. Immunopathol. 32, 157–171. Retrieved April, 2014, from http://www.ncbi.nlm.nih.gov/pmc/articles/PMC2868096/

57.) da Silva J. The dynamics of HIV-1 adaptation in early infection. Genetics. 2012;190:1087–1099. Retrieved April, 2014, from http://www.genetics.org/content/190/3/1087.full

Supplemental Figures

Sup. Figure 1



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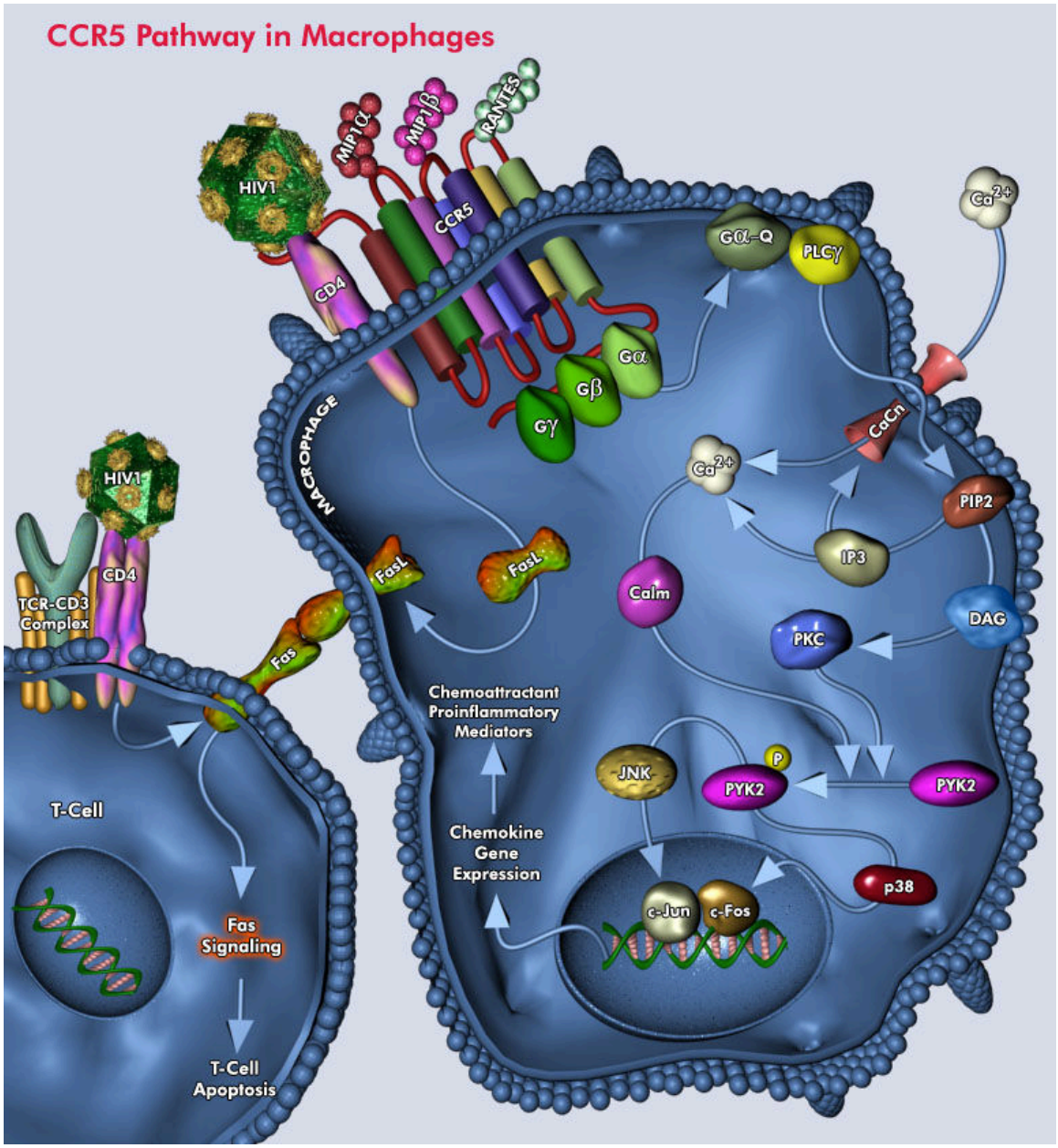

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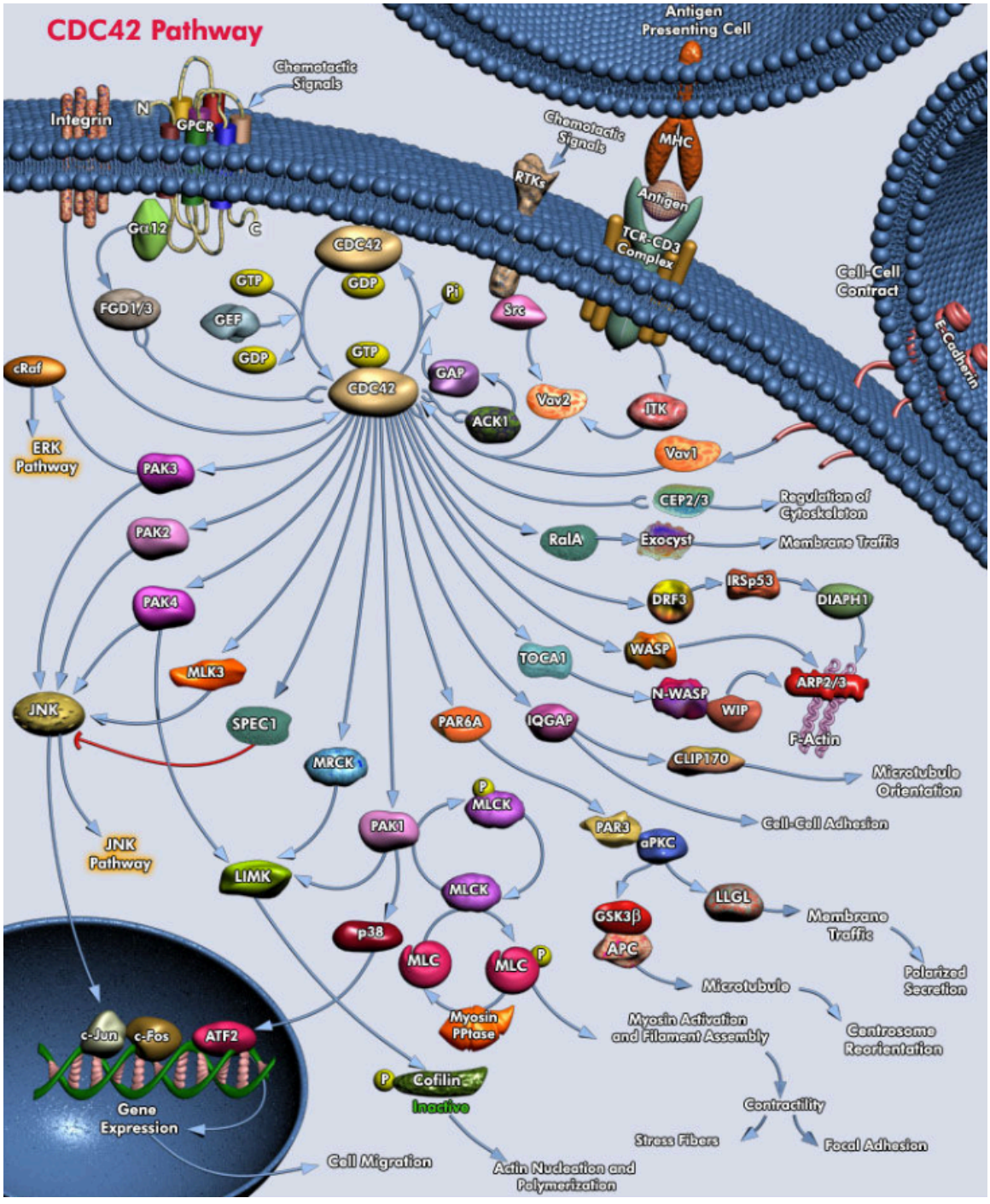

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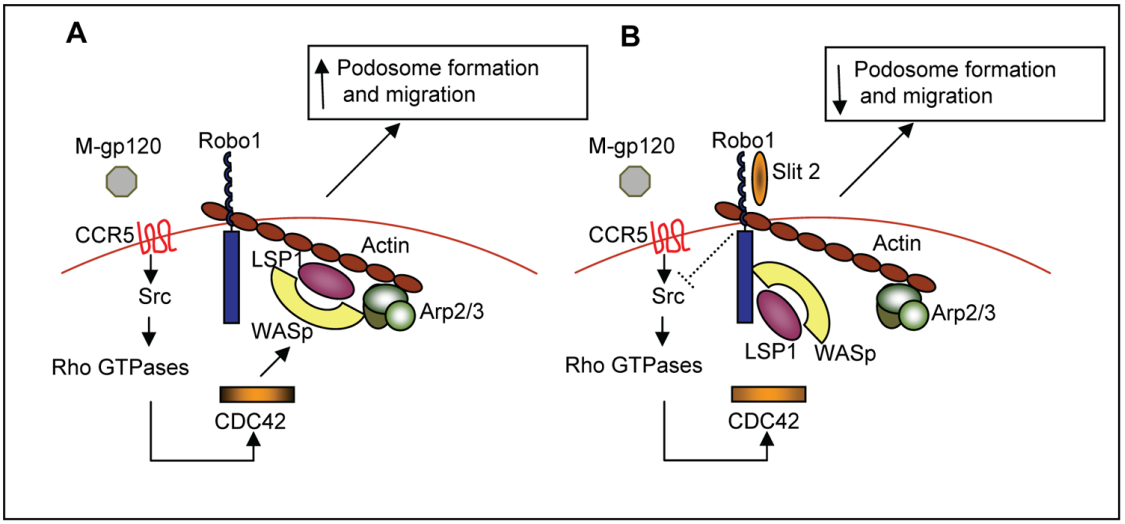

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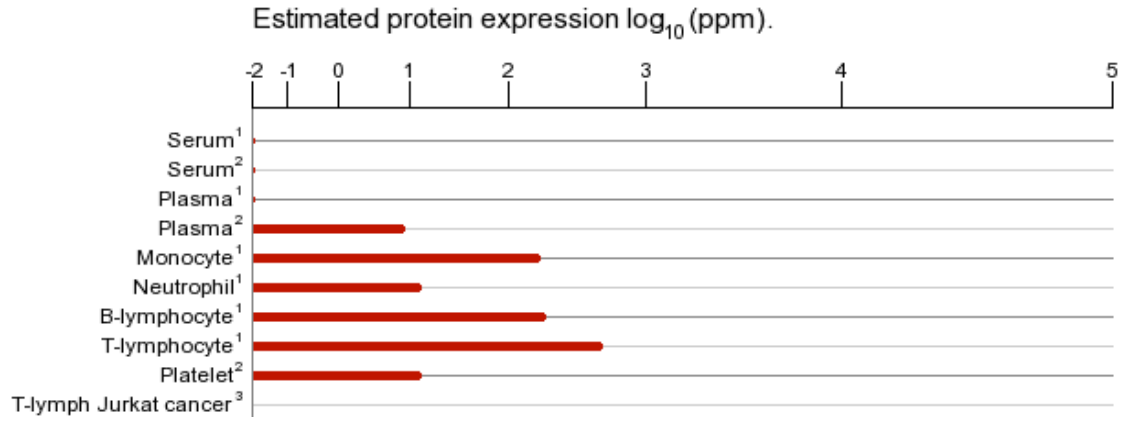
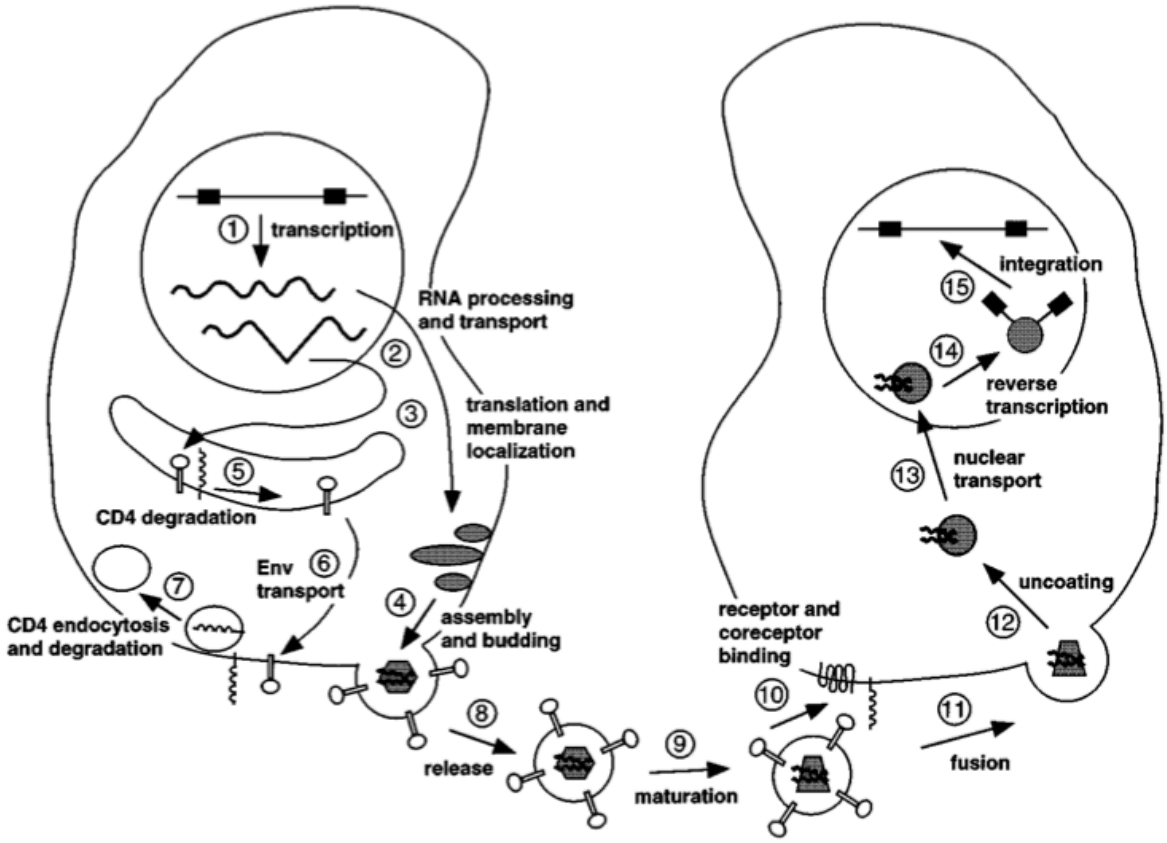
Sup. Figure 5



Estimated LSP1 Protein expression in different cell lineages.

Image from: http://www.genecards.org/cgi-bin/carddisp.pl?gene=LSP1

Sup. Figure 6



HIV-1 Replication Cycle, beginning from the Provirus stage (Step 1).

Image from: Frankel A, Young J. 1998.[10]